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doi:10.1289/ehp.7280 (available at http://dx.doi.org/)
Online 23 September 2004

The National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Department of Health and Human Services

Dietary fat interacts with PCBs to induce changes in lipid metabolism in LDL receptor deficient mice

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Acknowledgements: This study was supported in part by grants from NIEHS/NIH (ES 07380), USDA (2001-35200-10675), and the Kentucky Agricultural Experimental Station. This manuscript and its content are not associated with any conflict of interest.

Running Title: PCBs, Dietary Fat and Atherosclerosis

Key Words: dietary fat; PCB; lipid metabolism; gene expression; vascular endothelial cells; atherosclerosis

Abbreviations used: LDL-R, low density lipoprotein receptor; PCB, polychlorinated biphenyls; VCAM-1, vascular cell adhesion molecule-1; ROS, reactive oxidative species; BHT, butylated hydroxytoluene; BF_3, boron trifluoride; GC, gas chromatography; PBS, phosphate buffered saline; RNA, ribonucleic acid; RT-PCR, reverse transcription-polymerase chain reaction; CYP, cytochrome P450; SOD, superoxide dismutase; CPT, carnitine palmitoyltransferase; IL-6, interleukin-6;

ABSTRACT

There is evidence that dietary fat can modify the cytotoxicity of polychlorinated biphenyls (PCBs), and that coplanar PCBs can induce inflammatory processes critical in the pathology of vascular diseases. To test the hypothesis that the interaction of PCBs with dietary fat are dependent on the type of fat, LDL-R-/- mice were fed diets enriched either with olive oil or corn oil for 4 weeks. Half of the animals from each group were injected with PCB 77. VCAM-1 expression in aortic arches was non-detectable in the olive oil-fed mice, but was highly expressed in the presence of the PCB. PCB-treatment increased liver neutral lipids and decreased serum fatty acid levels only in mice fed the corn oil-enriched diet. PCB treatment increased mRNA expression of genes involved in inflammation, apoptosis and oxidative stress in all mice. Upon PCB treatment, mice in both olive and corn oil diet groups showed induction of genes involved in fatty acid degradation, however, with the upregulation of different key enzymes. Genes involved in fatty acid synthesis were only reduced upon PCB treatment in corn oil-fed mice, whereas lipid transport/export genes were altered in olive-oil fed mice. These data suggest that dietary fat can modify changes in lipid metabolism induced by PCBs in serum and tissues. These findings have implications for understanding the interactions of nutrients with environmental contaminants on the pathology of inflammatory diseases such as atherosclerosis.

INTRODUCTION

From epidemiological studies, there is substantial evidence that cardiovascular diseases are linked to environmental pollution and that exposure to polycyclic and/or polyhalogenated aromatic hydrocarbons can lead to human cardiovascular toxicity. For example, there was a significant increase in mortality from cardiovascular diseases among Swedish capacitor manufacturing workers exposed to PCBs for at least five years (Gustavsson and Hogstedt 1997), and most excess deaths were due to cardiovascular disease in power workers exposed to phenoxy herbicides and PCBs in waste transformer oil (Hay and Tarrel 1997). The increased prevalence of atherosclerosis may be associated with the ability of PCBs to modulate plasma and tissue lipids, events which can result in compromised lipid metabolism and lipid-dependent cellular signaling pathways. In a study with rhesus monkeys, a causal relationship between plasma lipid changes and PCB intake was observed following oral exposure of Aroclor 1254 (Bell et al. 1994). Moreover, a report by Tokunaga et al. (1999) confirms many other studies with chronic Yusho patients (accidental ingestion of rice-bran oil contaminated with PCBs), which showed in this population that elevated serum levels of triglycerides and total cholesterol were significantly associated with the blood PCB levels. Serum lipids also were shown to be affected by PCBs, which apparently can modify the regulatory mechanisms of synthesis and degradation of cholesterol (Jenke 1985). A major route of exposure to PCBs in humans is via oral ingestion of contaminated food products (Safe 1994). Therefore, circulating environmental contaminants derived from diets, such as PCBs, are in intimate contact with the vascular endothelium.

In addition to serum and vascular lipid changes, a number of studies have reported an increase in liver and hepatic microsomal lipids (total lipids, phospholipids, neutral lipids and cholesterol) following PCB administration (Garthoff et al.1977; Ishidate et al. 1978). We have shown previously that a single injection of PCB 77 resulted in a marked change in the fatty acid composition of rat hepatic microsomal fractions (Asais-Braesco et al. 1990). A study by Matsusue et al. (1999) has found that coplanar PCBs have a significant effect on the reduced synthesis of physiologically essential long-chain unsaturated fatty acids, such as arachidonic acid in rat liver, by suppressing delta 5 and delta 6 desaturase activities and thus allowing the omega-6 parent fatty acid, linoleic acid, to accumulate.

Little is known about the interaction of dietary fats and PCBs in the pathology of atherosclerosis. We have reported a significant disruption in endothelial barrier function when

cells were exposed to linoleic acid (reviewed in Hennig et al. 2001a). In addition to endothelial barrier dysfunction, another functional change in atherosclerosis is the activation of the endothelium that is manifested as an increase in the expression of specific cytokines and adhesion molecules. These cytokines and adhesion molecules are proposed to mediate the inflammatory aspects of atherosclerosis by regulating the vascular entry of leukocytes. We reported previously that coplanar PCBs and linoleic acid induce the expression of cytokines and adhesion molecules in cultured endothelial cells. In addition, both linoleic acid and PCB 77, and more markedly when applied in concert, can generate reactive oxidative species (ROS) that can trigger oxidative stress-sensitive pro-inflammatory signaling pathways (reviewed in Hennig et al. 2002a). These studies suggest that environmental contaminants like PCBs are atherogenic in part by their ability to alter endothelial cell lipid profile and metabolism and by inducing oxidative stress and proinflammatory genes.

Exposure to physiological concentrations of specific fatty acids, such as linoleic acid, can trigger inflammatory pathways leading to the upregulation of inflammatory cytokines (e.g., IL-6, IL-8) and adhesion molecules (e.g., VCAM-1, E-selectin). These genes initiate the chemoattraction and adhering of monocytes, events occurring early in the pathogenesis of atherosclerosis. The differential effect of various fatty acids is most likely due to different susceptibility to oxidation and thus generation of oxidative stress as well as their role in precursors of lipid derived second messengers (Hennig and Toborek 2001). Therefore, we hypothesize that selected dietary lipids may modulate the atherogenicity of environmental chemicals by interfering with metabolizing and inflammatory pathways and thus leading to dysfunction of the vasculature and related tissues.

Our present data indicate that dietary fat can modify changes in lipid metabolism induced by PCB in a LDL-receptor deficient mouse model, i.e., mice that develop atherosclerosis as a result of increased sensitivity to different types of dietary fat (Daugherty 2002). Our data also support our hypothesis that dietary oils rich in linoleic acid can further compromise gene expression during PCB cytotoxicity.

MATERIALS AND METHODS

Animal model and PCB treatment

The LDL-R-deficient mice used in this study were originally obtained from The Jackson Laboratory (Bar Harbor, ME; Stock Number: 002207) and bred at the University of Kentucky. LDL-R-/- mice have become a preferred model for atherosclerosis as their elevated LDL fraction resembles the lipoprotein profile of hypercholesterolemic humans (Daugherty and Whitman, 2002). All animal procedures were in compliance with the IACUC guidelines of the University of Kentucky. Mice were divided into four groups of five mice per treatment: olive oil-rich diet; olive oil-rich diet plus PCB injection; corn oil-rich diet; and corn oil-rich diet plus PCB injection. Mice were injected intraperitoneally with PCB 77 (170 μmoles/kg mouse) or the vehicle (olive oil or corn oil; Dyets Inc., Bethlehem, PA) at two times, i.e., at weeks one and three of the four-week feeding study.

After completion of the study, animals were euthanized using intraperitoneal ketamine injections. Serum, and aortic and liver tissues were obtained for analysis. According to our combined experience with several animal species, long-term intraperitoneal injections of 100 to 300 μmoles/kg body weight per injection are sufficient to initiate disease states, such as tumor promotion (Robertson et al. 1991). In our preliminary studies we saw adhesion molecule expression at 170 μmoles/kg mouse per injection; thus this concentration was chosen for the current study. This amount of PCB was based on calculated values from our *in vitro* experiments which were themselves based on levels that are usually found in humans after acute exposure (Jensen 1989; Wassermann et al. 1979).

Experimental diets

The oils were chosen due to previous cell culture work with individual fatty acids. In these experiments linoleic acid was able to amplify the inflammatory response of endothelial cells exposed to PCB 77. In addition, we have evidence that a high corn oil diet is proinflammatory and induces atherosclerotic pathology relative to a high olive oil diet (unpublished data). We therefore chose corn oil, which contains about 50% linoleic acid as triglycerides, and thus is a significant dietary source of linoleic acid. As a control, we chose olive oil, with the predominant fatty acid being oleic acid. Oleic acid is also an 18-carbon fatty acid but acted "neutral" when endothelial cells were coexposed to oleic acid and PCB 77. In fact,

our previous studies suggest that oleic acid has little effect or even can decrease an inflammatory response (Toborek et al. 2002).

Diets were custom prepared and vacuum packed (Dyets Inc., Bethlehem, PA). Diets were based on a modified AIN-76A purified rodent diet (Reeves 1997) with varying sources of fat. The dietary fat content, either olive oil or corn oil, was 150 g/kg total diet. The antioxidant content of each oil was adjusted by the manufacturer. The fatty acid composition in the different oils is shown in Figure 1.

Serum fatty acid analysis

Total plasma lipids were extracted by the method of Bligh and Dyer (Bligh and Dyer 1959) as modified by Williams *et al.* (Williams 1984). Internal standard (heneicosanoic acid, 5 μg in methanol) was added to the samples prior to lipid extraction. All solvents for liquid extraction contained 50 mg/L butylated hydroxytoluene (BHT) as an antioxidant (Silversand 1997). Lipids were dried under nitrogen followed by fatty acid esterification with BF_3-methanol. Fatty acid methyl esters were extracted with hexane for GC injection. The gas chromatograph (Agilent 6890 GC G2579A system, Agilent Technologies, Palo Alto, CA) was equipped with an OMEGAWAX^{TM} 250 capillary column. The following temperature program was used: 160°C for 5 min, an increase in temperature to 220°C at a rate of 2°C/min, followed by 220°C for 15 min. A model 5973 mass-selective detector (Agilent Technologies, Palo Alto, CA) was used for detection of separated lipids.

Neutral lipid staining of liver tissues

Liver sections were fixed overnight in 4% paraformaldehyde in PBS before embedding in OCT. Serial (10 μm) sections were mounted on MicroProbe slides, and neutral lipids were stained with Oil Red O, as described previously (Daugherty et al. 2000).

Immunostaining of aortic tissue

Aortic tissue from the thoracic regions was excised, immersed in OCT embedding medium, frozen at -20 °C, and 8 μm sections were cut on a cryostat. Immunocytochemistry was performed as described previously (Daugherty et al. 2000). Briefly, endogenous peroxidase was inactivated using hydrogen peroxide (3%) in methanol. Samples were blocked in the serum of

the secondary antibody host. Primary antibodies for VCAM-1 (PharMingen, San Diego, CA) were detected using biotinylated secondary antibodies and peroxidase ABC kits (Vectastain, Burlingame, CA). Aminoethylcarbozole was used as chromogen, and sections were counterstained with hematoxylin.

Gene expression analysis

For microarray analysis, total RNA was isolated from snap frozen liver tissue using RNAeasy (Quiagen, Valencia, CA). RNA samples were pooled for analysis of two data sets per treatment group. RNA integrity analysis and biotin-labeling of cRNA was performed by the Microarray Core Facility at the University of Kentucky. Labeled RNA was spotted on Murine Genome MOE 430 chips (Affymetrix, Santa Clara, CA) and detected in the Affymetrix 428 Fluorescence Reader.

Microarray data were confirmed by conventional RT-PCR. RNA was isolated from liver samples. cDNA was generated by reverse transcription and amplified by polymerase chain reaction using the following primers: CYP1A1 forward: 5' CAGATGATAAGGTCATCACGA 3', reverse: 5' TTGGGGATATAGAAGCCATTC 3', acetyl-CoA carboxylase forward: 5' ACAGTGAAGGCTTACGTCTG 3', reverse: 5' AGGATCCTTACAACCTCTGC 3' and β-actin forward: 5' ATGGATGACGATATCGCT 3', reverse: 5' ATGAGGTAGTCTGTCAGGT 3'. PCR products were separated on a 2% agarose gel and stained with SYBR gold and visualized using a phosphoimager (Fuji FLA-5000, Stamford, CT).

Quantitations and statistical analyses

Numeric data were analyzed using SYSTAT 7.0 (SPSS, Inc., Chicago, IL). Comparisons between treatments were made by one-way ANOVA with post-hoc comparisons of the means made by Bonferroni least significance difference procedure. Student t-tests were employed to compare gene expression data showing a PCB dependent change. Statistical probability of $P<0.05$ was considered significant.

Photomicrographs of VCAM-1 and neutral lipid staining in aortic roots and livers, respectively, were evaluated by individuals that were blinded to the specimen identification.

RESULTS

PCB treatment increases diet-dependent clearance of serum fatty acids

As expected, feeding a diet enriched with olive oil or corn oil resulted in serum fatty acid profiles (Figure 2) comparable to the fatty acid profile in the respective oils (Figure 1). PCB treatment had little effect on fatty acid patterns in animals fed the olive oil diet. In contrast, PCB treatment of corn oil-fed mice resulted in marked decreases in major serum fatty acids, with a quantitatively most significant serum clearance of serum linoleic acid.

PCBs increase neutral lipid staining in liver tissue

Baseline or control lipid staining (Oil Red O) appeared to be similar in liver tissues from both olive oil and corn oil fed mice. In contrast to the olive oil group, PCB exposure further increased neutral lipid staining only in LDL-R-/- mice fed the corn oil-enriched diet (Figure 3).

VCAM-1 expression is affected by diet and PCBs

VCAM-1 expression was negligible in mice fed the olive oil-enriched diet (Figure 4A), whereas, corn oil-fed mice exhibited elevated VACM-1 expression (Figure 4C). In corn oil-fed mice, PCB treatment further increased VCAM-1 staining in aortic tissues (Figure 4D). PCB treatment markedly increased VCAM-1 expression at the vascular surface in all animals, independent of dietary fat. Interestingly, PCB treatment increased VCAM-1 expression in smooth muscle-rich areas of the vessel in mice fed the corn oil-enriched diet (Figure 4D). This phenomenon was not observed in mice fed the olive oil-enriched diet.

Gene expression change in response to PCB77 in mice fed a high corn or high olive oil diet

PCB treatment markedly increased expression of selected genes involved in inflammation, apoptosis and oxidative stress in both diet groups (Table 1). Data represent expression values of both dietary groups compared with both dietary groups receiving PCBs. The oil-dependent effect of PCB 77 was most apparent in mRNA levels of genes involved in lipid metabolism (Table 2). Feeding diets rich in either corn or olive oil induced fatty acid degradation, however, with the upregulation of different key enzymes. For example, PCB treatment induced carnitine palmitoyltransferase (CPT) in corn oil-fed animals while glycerol-3-P-dehydrogenase and fatty acid CoA ligase 4 were induced in olive oil-fed mice. Genes involved in fatty acid synthesis,

such as acetyl-CoA-carboxylase and elongation of long chain fatty acids were only reduced by PCB 77 in corn oil-fed mice whereas lipid transport/export genes such as fatty acid binding protein 2 and 4, ATP-binding cassette A1 and apolipoprotein A-IV were altered in olive oil-fed mice in response to PCBs.

Microarray analysis of selected genes was confirmed by conventional RT-PCR. For example, PCB treatment only decreased expression of the acetyl CoA carboxylase gene in mice fed the corn oil diet (Figure 5A). As expected, PCB treatment increased CYP1A1 gene expression in all mice (Figure 5B).

DISCUSSION

There is substantial evidence that environmental pollutions can be correlated with the incidence of cardiovascular diseases (reviewed in Hennig et al. 2001b). This might be due to a PCB-mediated impairment of lipid metabolism. In the vasculature, alterations in lipid profile and lipid metabolism as a result of exposure to PCBs may be important components of endothelial cell dysfunction (reviewed in Hennig et al. 2002a). Endothelial cell dysfunction is an important factor in the overall regulation of vascular lesion pathology. We have reported recently that PCB 77 can increase expression of cytokines, such as interleukin-6 (IL-6), and adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), in cultured endothelial cells (Hennig et al. 2002b). Little is known about the interaction of dietary fats and PCBs in the pathology of atherosclerosis. We hypothesize that selected dietary lipids, and especially oils rich in linoleic acid, may increase the atherogenicity of environmental chemicals, such as PCBs, by cross-amplifying mechanisms leading to dysfunction of the vasculature and related tissues. Indeed, immunohistochemistry data from the current study demonstrate the cumulative effect of corn oil and PCB 77 on aortic VCAM-1 expression. While olive oil-fed mice did not show expression of this adhesion molecule unless they were injected with PCBs, corn oil feeding alone already resulted in a strong staining for VCAM-1. In corn oil-fed mice injected with PCBs, VCAM-1 expression could even be detected in the subendothelial space, suggesting a progressed state of atherosclerosis with adhesion molecule expression on smooth muscle cells. These data are in agreement with epidemiological studies that suggest diets high in olive oil or oleic acid protect against cardiovascular diseases (Massaro and De Caterina 2002).

However, the interaction of different dietary fats with environmental contaminants and the effect on the pathogenesis of atherosclerosis is unknown and has not been studied in LDL-R-/- mice.

There is considerable evidence that exposure to PCBs can lead to lipid changes in plasma and tissues and that this may be linked to lipophylic properties of PCBs and their interaction with lipids and especially with fatty acids. For example, exposure to Aroclor 1242 modified adipose tissue fatty acids, with a decrease of highly unsaturated fatty acids and an increase in monounsaturated fatty acids in membrane phospholipids (Kakela and Hyvarinen 1999). Our microarray analysis of liver mRNA suggests that PCB-lipid interactions are dependent on the type of dietary fat. For example, the PCB-mediated upregulation of genes involved in fatty acid uptake and catabolism, as well as down-regulation of genes involved in fatty acid synthesis involved different key enzymes depending on the oil that was used in the diet. It appears that PCBs had more effect on fatty acid synthesis in corn oil-fed animals, whereas there was a greater change in genes involved in fatty acid transport in olive oil-fed mice.

Overall, lipid metabolism was effected to a greater extend in corn oil-fed animals as demonstrated also by serum and liver lipid analyses. Lipids appear to be removed from the plasma and accumulate in tissues in corn oil-fed animals receiving PCB injection. A number of studies have reported an increase in liver and hepatic microsomal lipids (total lipids, phospholipids, neutral lipids and cholesterol) following PCBs administration (Asais-Braesco et al. 1990; Garthoff et al. 1977; Hinton et al. 1978; Ishidate et al. 1978; Robertson et al. 1991). The amplified toxicity of linoleic acid and PCBs to endothelial cells could thus be mediated by cellular accumulation of this fatty acid and its subsequent transformation to toxic cytotoxic epoxide metabolites (Viswanathan et al. 2003). Due to the very low basal activity of endothelial cell delta 6-desaturase, arachidonic acid is not produced from linoleic acid significantly in this type of cell (Spector et al. 1981; Debry and Pelletier 1991), which can result in linoleic acid accumulation within endothelial cells (Spector et al. 1981; Hennig and Watkins 1989). Furthermore, Matsusue et al. (1999) demonstrated that coplanar PCBs can suppress delta 5 and delta 6 desaturase activities. The decreased expression of the long chain fatty acid elongase detected in corn oil-fed mice treated with PCBs also suggests an impairment in fatty acid metabolism. Using endothelial cell culture models, we showed previously that linoleic acid uptake and cellular accumulation of this fatty acid is markedly increased in the presence of PCB

77, further supporting our hypothesis that PCB-induced endothelial cell dysfunction can be modulated by the cellular lipid milieu (Slim et al. 2001).

In summary, our data clearly demonstrate a selective interaction of diet, and especially dietary fats, with PCB-induced cellular functions. These findings may contribute to a better understanding of the interactive mechanisms of dietary fats and environmental contaminants as mediators of vascular endothelial cell dysfunction and vascular pathologies such as atherosclerosis.

REFERENCES

Asais-Braesco V, Macaire JP, Bellenand P, Robertson LW, Pascal G. 1990. Effects of two prototypic polychlorinated biphenyls (PCBs) on lipid composition of rat liver and serum. J Nutr Biochem 1: 350-354.

Bell FP, Iverson F, Arnold D, Vidmar TJ. 1994. Long-term effects of Aroclor 1254 (PCBs) on plasma lipid and carnitine concentrations in rhesus monkey. Toxicology 89: 139-153.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Med Sci 37: 911-917.

Daugherty A. 2002. Mouse models of atherosclerosis. Am J Med Sc. 323: 3-10.

Daugherty A, Manning MW, Cassis LA. 2000. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. J Clin Invest 105: 1605-1612.

Debry G, Pelletier X. 1991. Physiological importance of omega-3/omega-6 polyunsaturated fatty acids in man. An overview of still unresolved and controversial questions. Experientia 47: 172-178.

Garthoff LH, Friedman L, Farber TM, Locke KK, Sobotka TJ. Green S, Hurley NE, Peters EL, Story GE, Moreland FM, Graham CH, Keys JE, Taylor MJ, Scalera JV, Rothlein JE, Marks EM, Cerra FE, Rodi SB, Sporn EM. 1977. Biochemical and cytogenetic effects in rats caused by short-term ingestion of Aroclor 1254 or Firemaster BP6. J Toxicol Environ Health. 3: 769-796.

Gustavsson P, and Hogstedt C. 1997. A cohort study of Swedish capacitor manufacturing workers exposed to polychlorinated biphenyls (PCBs). Am J Ind Med 32: 234-239.

Hay A, and Tarrel J. 1997. Mortality of power workers exposed to phenoxy herbicides and polychlorinated biphenyls in waste transformer oil. Ann N Y Acad Sci 837: 138-156.

Hennig B, Hammock BD, Slim R, Toborek M, Saraswathi V, Robertson LW. 2002a. PCB-induced oxidative stress in endothelial cells: modulation by nutrients. Int J Hyg Environ Health 205: 95-102.

Hennig B, Toborek M. 2001. Nutrition and endothelial cell function: implications in atherosclerosis. Nutr Res 21: 279-293.

Hennig B, Toborek M, and McClain, CJ. 2001a. High-energy nutrients, fatty acids and endothelial cell function: implications in atherosclerosis. J Am Coll Nutr 20: 97-105.

Hennig B, Slim R, Toborek M, Hammock B, Robertson LW. 2001b. PCBs and Cardiovascular Disease: Endothelial Cells as a Target for PCB Toxicity. In: PCBs: Recent Advances in Environmental Toxicity and Health Effects (LW Robertson and LG Hansen, Eds.). Lexington, KY: The University Press of Kentucky, 211-220.

Hennig B, Meerarani P, Slim R, Toborek M, Daugherty A., Silverstone AE, Robertson LW. 2002b. Proinflammatory properties of coplanar PCBs: in vitro and in vivo evidence. Toxicol Appl Pharmacol 181: 174-183.

Hennig B, Watkins BA. 1989. Linoleic acid and linolenic acid: effect on permeability properties of cultured endothelial cell monolayers. Am J Clin Nutr 49: 301-305.

Hinton DE, Glaumann H, Trump BF. 1978. Studies on the cellular toxicity of polychlorinated biphenyls (PCBs). I. Effects of PCBs on microsomal enzymes and on synthesis and turnover of microsomal and cytoplasmic lipids. Virchows Arch B Cell Path 27: 279-306.

Ishidate K, Yoshida M, Nakazawa Y. 1978. Effect of typical inducers of microsomal drug-metabolizing enzymes on phospholipid metabolism in rat liver. Biochem Pharmacol 27: 2595-2603.

Jenke HS. 1985. Polychlorinated biphenyls interfere with the regulation of hydroxymethylglutaryl-coenzyme A reductase activity in rat liver via enzyme-lipid interaction and at the transcriptional level. Biochim Biophys Acta 837: 85-93.

Jensen AA. 1989. Background levels in humans. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products. (R. D. Kimbrough, and A. A. Jensen, Eds.). Amsterdam: Elsevier Science, 345-364.

Kakela R, Hyvarinen H. 1999. Fatty acid alterations caused by PCBs (Aroclor 1242) and copper in adipose tissue around lymph nodes of mink. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 122: 45-53.

Massaro M, De Caterina R. 2002. Vasculoprotective effects of oleic acid: epidemiological background and direct vascular antiatherogenic properties. Nutr Metab Cardiovasc Dis. 12: 42-51.

Matsusue K, Ishii Y, Ariyoshi N, Oguri K. 1999. A highly toxic coplanar polychlorinated biphenyl compound suppresses Delta5 and Delta6 desaturase activities which play key roles in arachidonic acid synthesis in rat liver. Chem Res Toxicol 12, 1158-1165.

Reeves PG. 1997. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127(5 Suppl): 838S-841S.

Robertson LW, Silberhorn EM, Glauert HP, Schwarz M, Buchmann A. 1991. Do structure-activity relationships for the acute toxicity of PCBs and PBBs also apply for induction of hepatocellular carcinoma? Environm Toxicol Chem 10: 715-726.

Safe S. 1994. Polychlorinated biphenyls (PCBs)- environmental impact, biochemical and toxic responses and implications for risk assessment. Crit Rev Toxicol 24: 87-149.

Silversand C, Haux C. 1997. Improved high-performance liquid chromatographic method for the separation and quantification of lipid classes: application to fish lipids. J Chromatogr B Biomed Sci Appl. 703: 7-14.

Slim R, Hammock BD, Toborek M, Robertson LW, Newman JW, Morisseau CH, Watkins BA, Saraswathi V, Hennig B. 2001. The role of methyl-linoleic acid epoxide and diol metabolites in the amplified toxicity of linoleic acid and polychlorinated biphenyls to vascular endothelial cells. Toxicol Appl Pharmacol 171: 184-193.

Spector AA, Kaduce TL, Hoak JC, Fry GL. 1981. Utilization of arachidonic and linoleic acids by cultured human endothelial cells. J Clin Invest 68: 1003-1011.

Toborek M, Lee YW, Garrido R, Kaiser, S, Hennig B. 2002. Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells. Am J Clin Nutr 75: 119-125.

Tokunaga S, Hirota Y, Kataoka K. 1999. Association between the results of blood test and blood PCB level of chronic Yusho patients twenty five years after the outbreak. Fukuoka Igaku Zasshi. 90: 157-161.

Viswanathan S, Hammock BD, Newman JW, Meerarani P, Toborek M, and Hennig B. 2003. Involvement of CYP 2C9 in mediating the proinflammatory effects of linoleic acid in vascular endothelial cells. J Am Coll Nutr 22: 502-510.

Wassermann M, Wassermann D, Cucos S, and Miller HJ. 1979. World PCBs map: storage and effects in man and his biologic environment in the 1970s. Ann N Y Acad Sci 320: 69-124.

Williams RD, Wang E, Merrill AH Jr. 1984. Enzymology of long-chain base synthesis by liver: characterization of serine palmitoyltransferase in rat liver microsomes. Arch Biochem Biophys. 228: 282-291.

FIGURE LEGENDS

Figure 1: Fatty acid analysis of the two oils used in the feeding study. Only the major fatty acids of interest are listed. Fatty acids are listed as g/100 g total fatty acids. 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid.

Figure 2: Fatty acid profile in serum. Serum was prepared by centrifugation of whole blood. Samples were filtered and vials were crimped under nitrogen. Fatty acids from 5 μL serum were separated by HPLC and detected by MS. Samples are means ± SEM (n-5). *Significantly different from respective diet treatment (without PCBs). 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid.

Figure 3: Lipid staining of liver sections. Liver sections were fixed overnight in 4% paraformaldehyde in PBS before embedding in OCT. Serial (10 μm) sections were mounted on MicroProbe slides and neutral lipids were stained with Oil Red O. The magnification was 200x. (A) olive oil-fed mice; (B) olive oil-fed mice injected with PCB 77; (C) corn oil-fed mice; (D) corn oil-fed mice injected with PCB 77.

Figure 4: Immunoreactivity of VCAM-1 antiserum against sections of mouse aortic arches. Primary antibodies for VCAM-1 were detected using biotinylated secondary antibodies and peroxidase ABC kits. The red color as seen in panels B, C and D reflects positive chromogen development for VCAM-1 immunostaining on the endothelial surface (panels B, C, D), as well as subendothelial tissue (panel D). The magnification was 400x. (A) olive oil-fed mice; (B) olive oil-fed mice injected with PCB 77; (C) corn oil-fed mice; (D) corn oil-fed mice injected with PCB 77.

Figure 5: mRNA expression of acetyl-CoA carboxylase (A) and cytochrome P450 1A1 (B) as analyzed by RT-PCR. mRNA was isolated from liver samples, and cDNA was generated by reverse transcription and amplified by polymerase chain reaction. Graphs show expression data from five mice (OO, olive oil; CO, corn oil), and the lower panels show one representative sample per treatment group of RT-PCR gels. Graphs are normalized to β-actin. Values are means ± SEM (n-5). *Significantly different from all other groups.

1 Table 1: PCB mediated upregulation of mRNA expression of selected genes involved in
2 inflammation, apoptosis and oxidative stress.

Inflammation	**High fat diets** (Mean ± SEM)	**High fat diets + PCB** (Mean ± SEM)	**P-value**
Neuronal pentraxin	33.0 ± 5.3	118.2 ± 8.2	P < 0.01
Amyloid beta (A4) precurser	1315.6 ± 3.0	1954.6 ± 2.0	P < 0.12
Interleukin 6 signal transducer	129.6 ± 31.3	229.4 ± 20.5	P < 0.04
Interleukin 2 receptor, gamma chain	177.9 ± 28.6	331.1 ± 42.5	P < 0.02
Matrix metalloproteinase 19	95.8 ± 23.9	124.95 ± 28.9	P < 0.47
Membrane metalloendopeptidase	110.9 ± 13.2	159.13 ± 30.0	P < 0.19
Apoptosis			
Caspase 6	490.9 ± 67.7	703.5 ± 41.6	P < 0.04
Caspase 7	183.8 ± 40.6	326.9 ± 1.9	P < 0.01
Caspase 8 and FADD-like	79.4 ± 13.0	134.0 ± 32.5	P < 0.17
Apoptosis inhibitor 5	83.7 ± 11.5	147.9 ± 13.6	P < 0.01
Oxidative Stress			
Cytochrome P450, 1A1	692.1 ± 465.0	2999.8 ± 691.1	P < 0.03
Cytochrome P450, 1A2	8823.9 ± 2118.8	16927.4 ± 979.0	P < 0.01
NADPH oxidase 4	625.8 ± 150.4	844.5 ± 50.6	P < 0.22
Superoxide dismutase 2	125.1 ± 15.6	204.9 ± 18.3	P < 0.02

3

Table 2: Relative expression changes of genes involved in lipid metabolism upon PCB 77. Data shown refer to ratios of diet alone compared with diet plus PCB 77 within each dietary treatment: − no change; ↑ and ↓ ≥ 1.5 fold change; ↑↑ and ↓↓ ≥ 2 fold change.

Gene	Function	Olive Oil	Corn Oil
Carnitine palmitoyl-transferase 1	Fatty acid degradation	-	↑↑
Glycerol-3-P-dehydrogenase	Fatty acid degradation	↑↑	-
Fatty acid CoA ligase 4	Fatty acid degradation	↑↑	-
Acetyl-coenzyme A carboxylase beta	Fatty acid synthesis	-	↓↓
Long chain fatty acyl elongase	Fatty acid elongation	-	↓↓
CD 36	Fatty acid uptake	-	↑
Fatty acid binding protein 4	Fatty acid transport	↓	-
Fatty acid binding protein 2	Fatty acid transport	↓↓	-
ATP-binding cassette A1	Cholesterol export	↓↓	-
Apolipoprotein A-IV	Lipoprotein metabolism	↑↑	-
HDL binding protein	Lipoprotein metabolism	↓↓	-
Cytochrome P450 1A1	Fatty acid metabolism	↑↑	↑↑

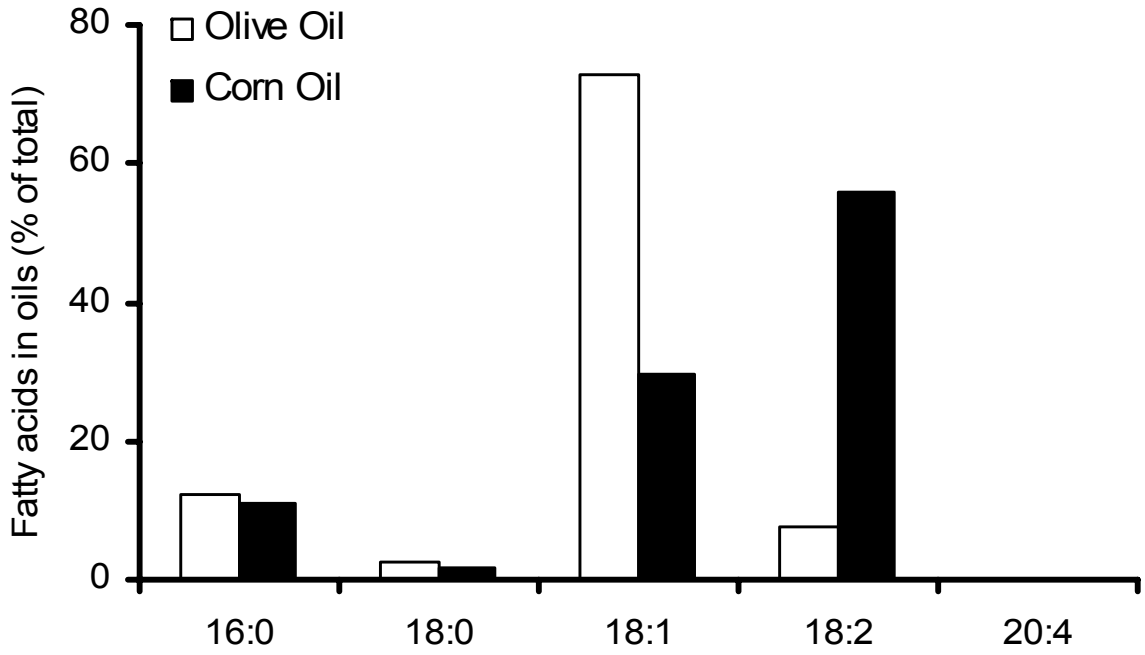
80
60
40
20
0
Olive Oil
Corn Oil
Fatty acids in oils (% of total)
16:0
18:0
18:1
18:2
20:4

Figure 1

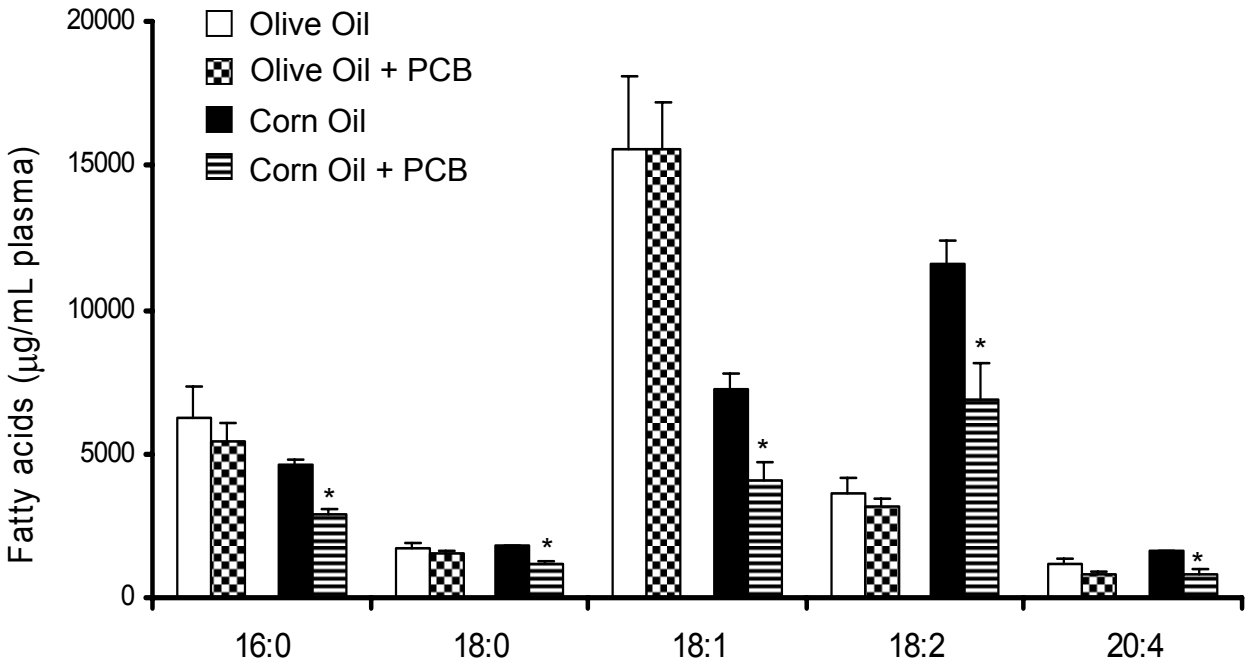
Olive Oil
Olive Oil + PCB
Corn Oil
Corn Oil + PCB
Fatty acids (µg/mL plasma)
20000
15000
10000
5000
0
16:0
18:0
18:1
18:2
20:4
*

Figure 2

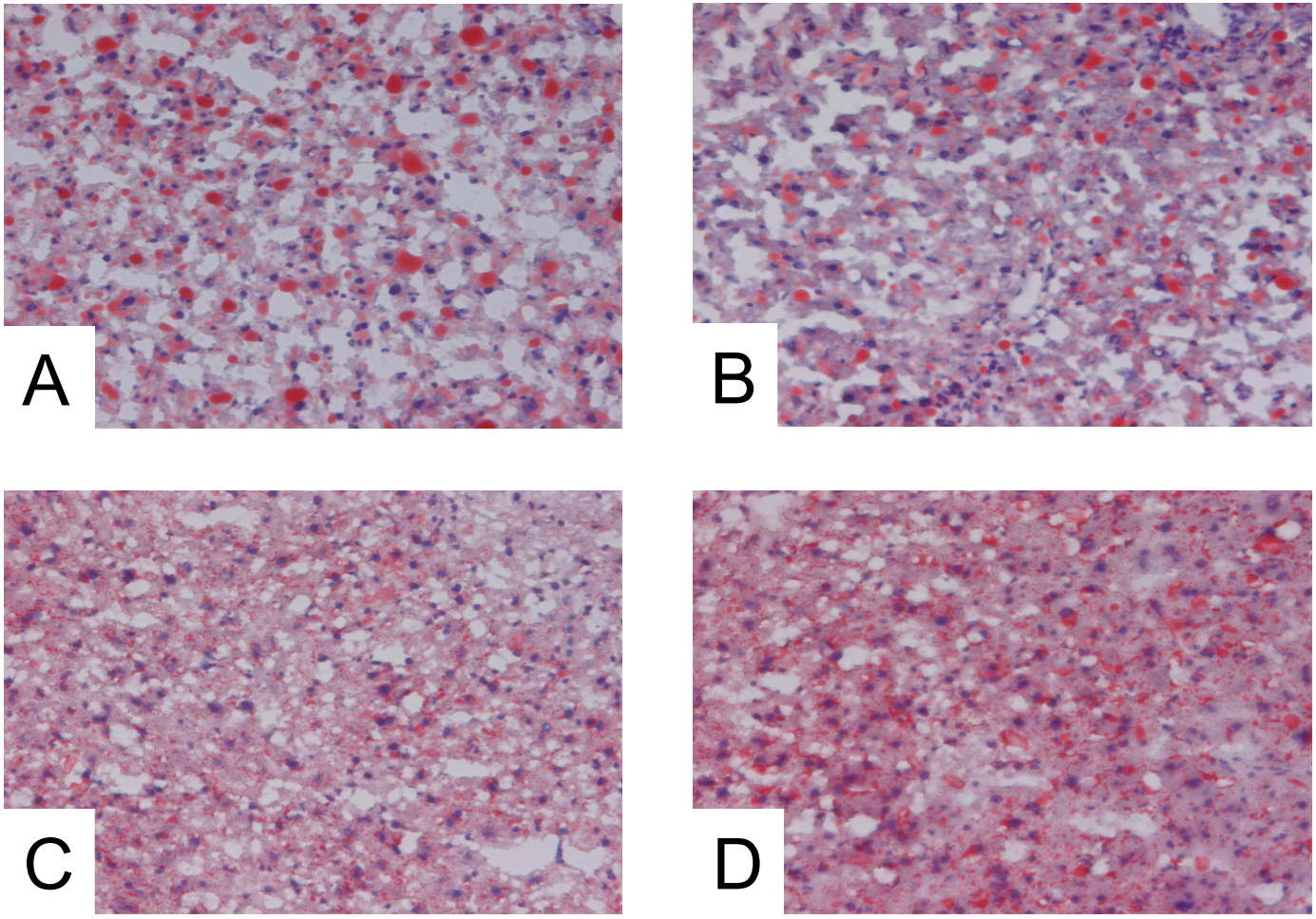

Figure 3

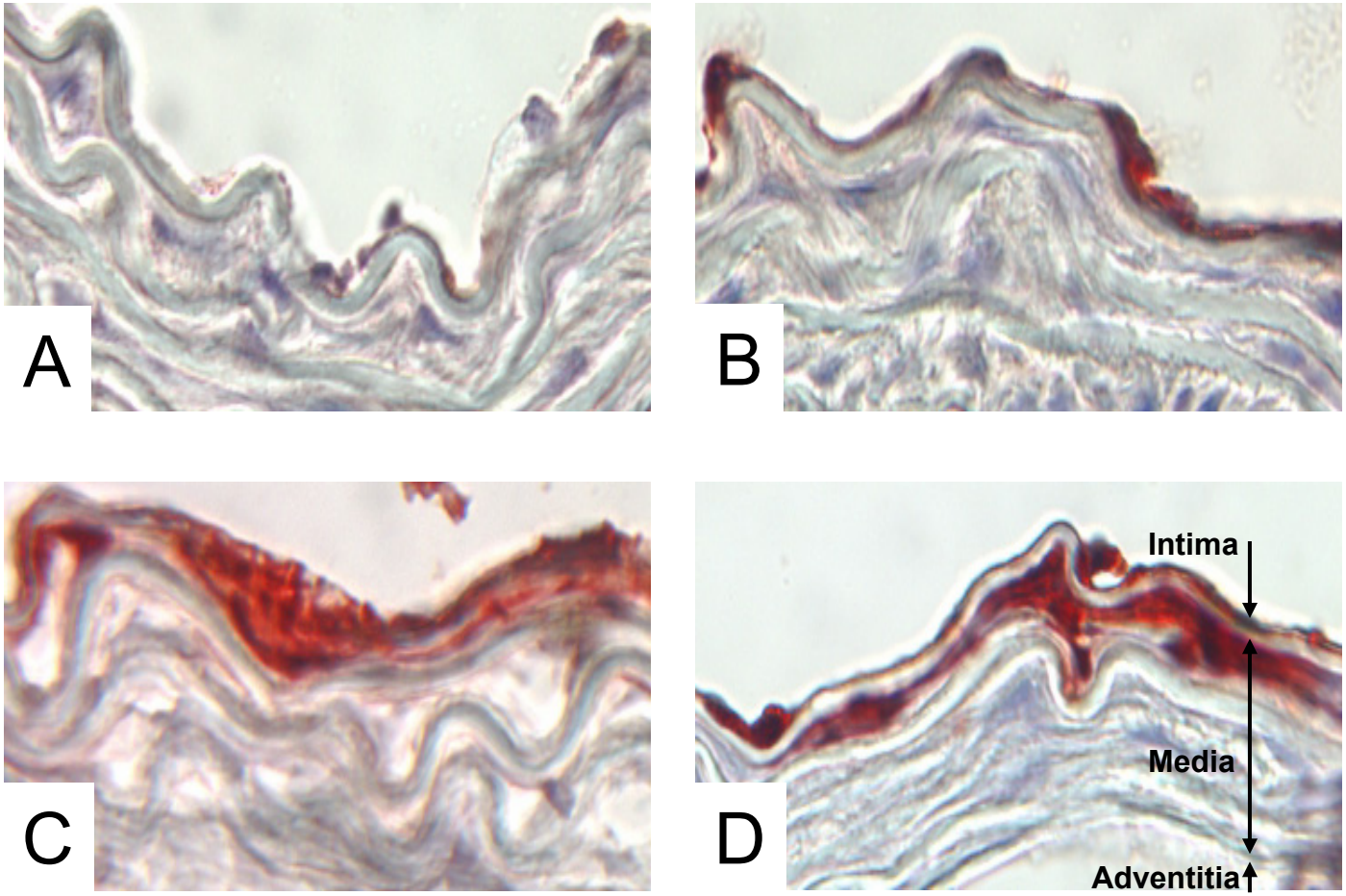

Figure 4

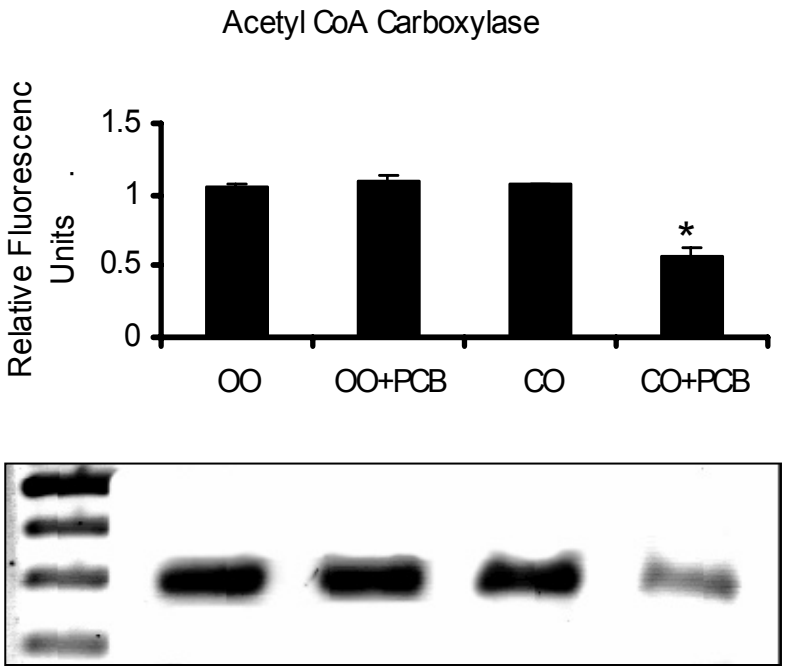

Figure 5A

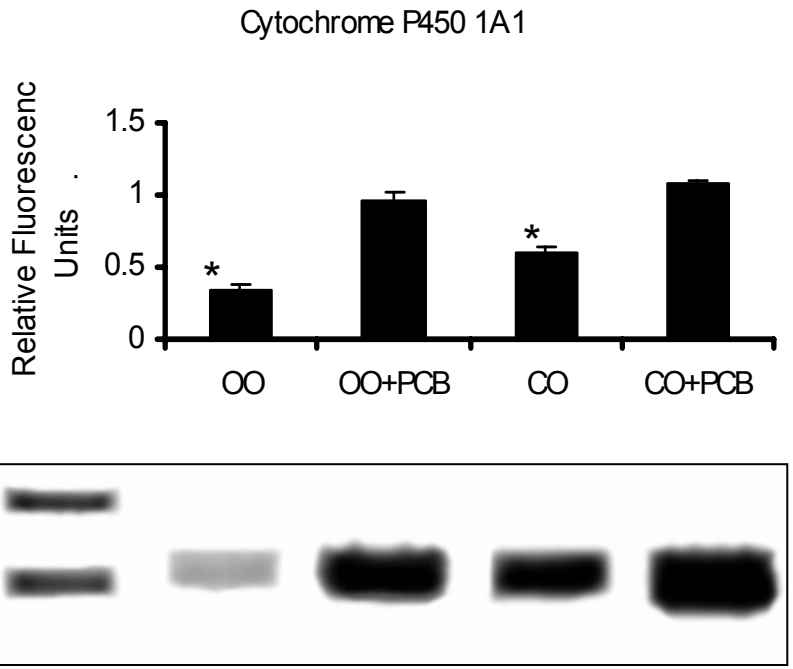

Figure 5B